

A PROCESS FOR DECREASING THE AMOUNT OF CHOLESTEROL
IN A MARINE OIL USING A VOLATILE WORKING FLUID

FIELD OF THE INVENTION

This invention relates to a process for decreasing
5 the amount of cholesterol in a mixture comprising a ma-
rine oil, containing the cholesterol. The present inven-
tion also relates to a volatile cholesterol decreasing
working fluid, a health supplement and a pharmaceutical,
prepared according to the process mentioned above.

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BACKGROUND OF THE INVENTION

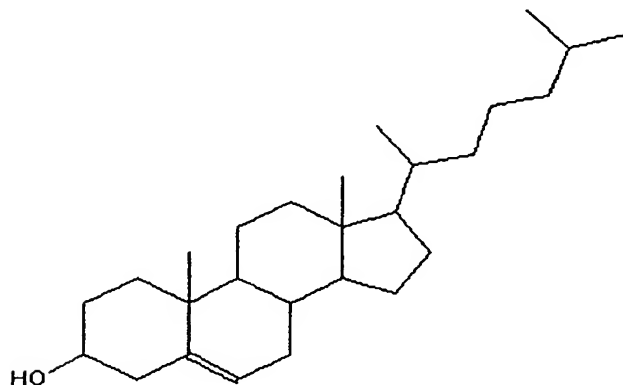
It is known that cholesterol is an important steroid
found in the lipids (fats) in the bloodstream and in all
body's cells in mammals. Cholesterol is used to form cell
15 membranes, some hormones and other needed tissues. A mam-
mal will get cholesterol in two ways; the body produces
some of it, and the rest comes from products that the
mammal consumes, such as meats, poultry, fish, eggs, but-
ter, cheese and whole milk. Food from plants like fruits,
20 vegetables and cereals do not include cholesterol.

Cholesterol and other fats can't dissolve in the
blood. They have to be transported to and from the cells
by special carriers called lipoproteins, named on basis
of their density. Low-density lipoprotein, or LDL, trans-
25 port cholesterol from the liver to peripheral tissues and
LDL transported cholesterol is known as the "bad" chole-
sterol, because too much LDL cholesterol can clog the ar-
teries to the heart and increase the risk of heart at-
tack. High-density lipoprotein, or HDL, transport choles-

terol back to the liver where surplus cholesterol is disposed of by the liver as bile acids. HDL transported cholesterol is known as the "good" cholesterol and high levels of HDL may reduce cholesterol deposits in arteries.

- 5 For an organism to remain healthy, there has to be an intricate balance between the biosynthesis of cholesterol and its utilization, so that arterial deposition is kept at a minimum.

In e.g. marine oils, cholesterol is stored as "free" respectively as "bound" cholesterol. In the bound form,
10 cholesterol is esterified on the OH-group by a fatty acid.



Structural formula of cholesterol

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The commercially important polyunsaturated fatty acids in marine oils, such as fish oil, are preferably EPA (eicosapentaenoic acid, C₂₀:5), DHA (docosahexaenoic acid, C₂₂:6). The full nomenclature of these acids according to the IUPAC system is: EPA cis-5,8,11,14,17-eicosapentaenoic acid, DHA cis-4,7,10,13,16,19-docosahexaenoic acid. For many purposes it is necessary that the marine oils should be refined in order to in-

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crease the content of EPA and/or DHA to suitable levels, or to reduce the concentrations of, or even eliminate, certain other substances which occur naturally in the raw oil, e.g. cholesterol.

5 The fatty acids EPA and DHA are also proving increasingly valuable in the pharmaceutical and food supplement industries in particular. It is also very important for fish oils and other temperature sensitive oils (i.e. oils that contains long chain polyunsaturated fatty
10 acids) to keep the load of the temperature as low as possible. Concerning the amount of cholesterol in the oils, it is specially a problem in fish oils and milk fat.

Further, as the link between high serum cholesterol levels and heart disease has become increasingly apparent,
15 cholesterol-free and cholesterol-reduced food products have become more attractive to consumers, and food products that have no or reduced cholesterol are gaining popularity as well as an increasing share of the market. Consequently, removal or reduction of cholesterol in high
20 cholesterol foods has the potential to substantially increase marketability and value.

The removal or reduction of cholesterol in marine oils is not a trivial matter. Several different techniques to accomplish this task have been developed, each
25 with varying levels of success. The content of cholesterol in marine oils will become a much more important parameter for the process industry in the future.

Some methods of treating a fish oil is known from the prior art. Such methods include conventional vacuum
30 steam distillation of fish oils at high temperatures which creates undesirable side reactions, decreases the content of EPA and DHA in the oil and the resulting prod-

uct has a poor flavour stability and poor resistance to oxidation.

SUMMARY OF THE INVENTION

5 One object of the invention is to offer an effective process for decreasing the amount of cholesterol in a mixture comprising a marine oil, containing the cholesterol, preferably by decreasing and separating the amount of cholesterol present in free form.

10 According to a first aspect of the invention, this and other objects are achieved with a process for decreasing the amount of cholesterol in a mixture comprising a marine oil, the marine oil containing the cholesterol, which process comprises the steps of adding a

15 volatile working fluid to the mixture, where the volatile working fluid comprises at least one of a fatty acid ester, a fatty acid amide and a hydrocarbon, and subjecting the mixture with the added volatile working fluid to at least one stripping processing step, in

20 which an amount of cholesterol in the marine oil is separated from the mixture together with the volatile working fluid. Most preferably, the amount of cholesterol present in the marine oil that is separated from the mixture together with the volatile working fluid is constituted by

25 cholesterol in free form. Herein, "an amount" is interpreted to include decreasing of an amount up to almost 100% of cholesterol present in free form, i.e. a substantial removal of cholesterol in free form from a marine fat or oil composition, at low mixture flow rates. The

30 content of bound cholesterol is less affected by the stripping process according to the invention, since cholesterol in bound form has a higher boiling point compared to the working fluid according to the invention.

The use of a volatile working fluid, where the volatile working fluid comprises at least one of a fatty acid ester, a fatty acid amide and a hydrocarbon, or any combination thereof, in a stripping process (or processing
5 step) for decreasing the amount of cholesterol present in a marine oil in free form has a number of advantages.

An advantage of using a volatile working fluid in a stripping process is that the cholesterol present in free form can more easily be stripped off together with the
10 volatile working fluid. Preferably, this is possible as long as the volatile working fluid is essentially equally or less volatile than the cholesterol that shall be removed from the oil mixture. The stripped cholesterol present in free form and the volatile working fluid will be
15 found in the distillate. When the volatile working fluid have the mentioned property, in combination with beneficial stripping process conditions, it is possible to separate, or strip off, almost all cholesterol present in a marine oil in free form more effectively. The effect of
20 adding a volatile working fluid to a marine oil mixture before stripping is larger and also more commercial useful, compared to a general process for decreasing cholesterol in an oil mixture, at higher flow rates. Herein, "high flow rates" is interpreted to include mixture flow
25 rate in the interval of 80-150 kg/h·m². Under the process conditions mentioned above, the use of a volatile working fluid open up for a much better utilization of the capacity of the process equipment and a more rapid stripping process.

30 Further, according to the present stripping process it is also possible to decrease an effective amount of cholesterol present in a marine oil in free form at lower temperatures, preferably at a temperature in the interval

of 150 - 220°C, compared to the techniques known from the prior art. It is especially important to keep the temperature as low as possible during processing of marine oils, such as fish oils, and other temperature accommodating oils (i.e. oils comprising long chains of polyunsaturated fatty acids). This is not so critical for oils not included above. In addition, the volatile working fluid according to the invention allows cholesterol present in free form to be stripped off by e.g. molecular distillation even from oils of lower quality, i.e. oil for feed purposes.

In a preferred embodiment of the present invention the volatile working fluid is an organic solvent or solvent mixture with a volatility comparable to the cholesterol in free form. The volatile working fluid of the present invention is at least one of a fatty acid ester, a fatty acid amide, and a hydrocarbon, also including any combinations thereof.

In another preferred embodiment the volatile working fluid comprises at least one fatty acid ester composed of C10-C22 fatty acids and C1-C4 alcohols, or a combination of two or more fatty acid ester each composed of C10-C22 fatty acids and C1-C4 alcohols. Preferably, the volatile working fluid is at least one of amides composed of C10-C22 fatty acids and C1-C4 amines, C10-C22 free fatty acids, and hydrocarbons with a total number of carbon atoms from 10 to 40. Most preferably, the volatile working fluid is a mixture of fatty acids from marine oils, e.g. fish body oil and/or fish liver oil, and/or ethyl or methyl esters of such marine fatty acids.

In another embodiment of the invention a volatile working fluid may be produced by subjecting fats or oils from an available source, for instance fats or oils

obtained from at least one of animal, microbial or vegetable origin, to an inter-esterification process, in which process the triglycerides in the fats or oils are converted into esters of aliphatic alcohols.

- 5 Additionally, a bio-diesel and/or a mineral oil can be used as a volatile working fluid. In the case when the volatile working fluid is a biodiesel, it can be produced by a process, which is in common use for production of engine fuels (biodiesel), and therefore also known by a
- 10 man skilled in the art, which process comprises mixing the fat or oil with a suitable amount of aliphatic alcohol, adding a suitable catalyst and heating the mixture for a period of time. Similar esters of aliphatic alcohols may also be produced by a high-temperature
- 15 catalytic direct esterification process reacting a free fatty acid mixture with the appropriate aliphatic alcohol. The fatty acid ester mixture produced in this manner may be used as a volatile working fluid as it is, but normally the conversion to esters of aliphatic
- 20 alcohols is not complete, the conversion process preferably leaving some un-reacted non-volatile glycerides in the mixture. Further, some fats or oils may also contain certain amounts of non-volatile, non-glyceride components (e.g. polymers). Such non-volatile
- 25 components will be transferred to, and mixed with the final product, which product is low in cholesterol, when the fatty acid ester mixture is used as working fluid. A working fluid produced in this manner should therefore be subjected to a distillation, preferably a molecular
- 30 and/or short path distillation, in at least one step, which distillation process generates a distillate more suitable to be used as a new volatile working fluid.

In addition, the volatile working fluid according to the invention allows cholesterol to be stripped off by e.g. molecular distillation even from oils of lower quality.

5 In another preferred embodiment of the process, at least one of a fatty acid ester and a fatty acid amide constituting said volatile working fluid is obtained from at least one of a vegetable, microbial and animal fat or oil, being edible or for use in cosmetics. Preferably,
10 the animal fat or oil is a marine oil, e.g. a fish oil or an oil from other marine organism e.g. sea mammals. It is also possible that the fatty acid esters mentioned above can e.g. be a byproduct from distillation of an ethyl ester mixture prepared by ethylation of preferably a fish
15 oil. In the process industry trade with intermediates is increasing and opens up for an extra financial income.

In fish oils cholesterol is typically present in concentrations of 5-10 mg/g, but higher concentrations have been observed. In this case 2-4 mg/g is typically
20 bound cholesterol and 3-6 mg/g is free cholesterol. Free cholesterol can be effectively removed by adding a volatile working fluid prior to at least one of the stripping processes according to the invention.

In another embodiment of the process according to
25 the invention, the marine oil containing saturated and unsaturated fatty acids in the form of triglycerides, and the marine oil is obtained from fish and/or sea mammals. Marine oils that contains no or reduced amounts of cholesterol present in free form are gaining popularity
30 as well as an increasing share of the market.

It is important to note that the invention is not limited to procedures where the working fluid is prepared from the same origin as the oil that is being purified.

In a preferred embodiment of the invention, the ratio of (volatile working fluid):(marine oil) is about 1:100 to 15:100. In a more preferred embodiment the ratio of (volatile working fluid):(marine oil) is about 3:100 to 8:100.

In a preferred embodiment of the invention, said stripping process step is carried out at temperatures in the interval of 120-270 °C.

In a most preferred embodiment, the stripping processing step is carried out at temperatures in the interval of 150-220 °C. By adding a volatile working fluid to the marine oil mixture at this temperatures the invention surprisingly shows that even termolabile polyunsaturated oils can be treated with good effect, without causing degradation of the quality of the oil.

In another preferred embodiment, the stripping processing step is carried out at a pressure below 1 mbar.

In further preferred embodiment, the stripping processing step is at least one of a thin-film evaporation process, a molecular distillation or a short-path distillation, or any combination thereof. If at least one stripping process step is a thin-film evaporation the process is also carried out at mixture flow rates in the intervall of 30-150 kg/h·m², most preferably in the range of 80-150 kg/h·m². The effect of adding a volatile working fluid to a marine oil mixture before stripping is larger and also more commercial useful, compared to a general process for decreasing cholesterol present in a marine fat or oil in free form at higher flow rates.

By using a stripping process, e.g. a distillation method, for decreasing the amount of cholesterol present

in a marine oil in free form, the marine oil mixture comprising a volatile working fluid, it is possible to carry out the stripping processes at lower temperatures, which spare the oil and is at the same time favourable to the end oil product.

Another embodiment of the present invention is a stripping process wherein a working fluid is added to a mixture comprising a marine oil, containing cholesterol, prior to a thin-film evaporation process, and the volatile working fluid comprises at least one of a fatty acid ethyl ester and a fatty acid methyl ester (or any combinations thereof), and subjecting the mixture with the added working fluid to a thin-film evaporation step, in which an amount of cholesterol present in free form in the marine oil is separated from the mixture together with the volatile working fluid.

In a preferred embodiment according to the invention the stripping process is carried out by a molecular distillation in the following intervals; mixture flow rates in the interval of 30-150 kg/h·m², temperatures in the interval of 120-270 °C and a pressure below 1 mbar.

In a most preferred embodiment of the invention the molecular distillation is carried out at temperatures in the interval of 150-220°C and at a pressure below 0,05 mbar, or by a thin-film process, which process is carried out at 80-150 kg/h·m² or at flow rates in the range of 800-1600 kg/h at a heated thin film area of 11 m²; 73-146 kg/h·m². Please note, that the present invention can also be carried out in one or more subsequent stripping processing steps.

In another preferred embodiment of the present invention, a volatile cholesterol decreasing working fluid, for use in decreasing an amount of cholesterol

present in a marine oil in free form, the volatile working fluid is comprising at least one of a fatty acid ester, a fatty acid amide and a hydrocarbon, with essentially equally or less volatility compared to the cholesterol that is to be separated from the marine oil, or any combination thereof.

Preferably, the volatile cholesterol decreasing working fluid is generated as a fractionation product. Additionally, the volatile cholesterol decreasing working fluid is a by-product, such as a distillation fraction, from a regular process for production of ethyl and/or methyl ester concentrates. This by-product according to the invention can be used in a new process preferably for fat or oil being edible or for use in cosmetics. More preferably, the volatile cholesterol decreasing working fluid, for use in decreasing an amount of cholesterol present in a marine fat or oil, can be a by-product (a distillate fraction) from a regular process for production of ethyl ester concentrates, wherein a mixture comprising an edible or a non-edible fat or oil, preferably a fish oil, is subjected to an ethylating process and preferably a two-step molecular distillation. In the two-step molecular distillation process a mixture consisting of many fatty acids on ethyl ester form is separated from each other in; a volatile (light fraction), a heavy (residuum fraction) and a product fraction. The volatile fraction from the first distillation is distilled once more and the volatile fraction from the second distillation process is then at least composed of the volatile working fluid, preferably a fatty acid ethyl ester fraction. This fraction consists of at least one of C14 and C16 fatty acids and at least one of the C18 fatty acids from the fat or oil, and is

therefore also compatible with the edible or non-edible oil. The fraction can be redistilled one or more times if that is deemed to be suitable. This prepared working fluid can then be used as a working fluid in a new
5 process for decreasing the amount of cholesterol present in a marine oil in free form, wherein the edible or non-edible fats or oils and the marine oil are of the same or different types.

In another preferred embodiment of the invention the
10 volatile working fluid comprises at least one of an ester and/or an amide composed of shorter fatty acids and longer alcohols or amines, or any combination thereof.

In a preferred embodiment of the invention, the volatile cholesterol decreasing working fluid, for use in
15 decreasing an amount of cholesterol present in a marine oil, is preferably a fatty acid ester (e.g. fatty acid ethyl ester or fatty acid methyl ester) or a fatty acid amide obtained from at least one of vegetable, microbial and animal fat or oil, or any combination thereof.
20 Preferably, said animal fat or oil is a marine oil, for instance a fish oil and/or an oil from sea mammals.

In another embodiment of the invention, a volatile cholesterol decreasing working fluid according to the present invention, is used in a process for decreasing
25 the amount of cholesterol in a mixture comprising a marine oil, the marine oil containing the cholesterol, in which process the volatile working fluid is added to the mixture and then the mixture is subjected to at least one stripping processing step, preferably a thin-film evapo-
30 ration process, a molecular distillation or a short-path distillation or any combination thereof, and in which process an amount of cholesterol present in the marine oil in free form is separated from the oil mixture

In a more preferred embodiment, the volatile cholesterol decreasing working fluid is a by-product, such as a distillate fraction, from a regular process for production of ethyl and/or methyl ester concentrates.

5 In another preferred embodiment a health supplement, or a pharmaceutical containing oil (end) products with a decreased amount of cholesterol, preferably strongly limited amounts of cholesterol present in free form, prepared according to at least one of the previously mentioned processes is disclosed. For the pharmaceutical and
10 food supplement industries, marine oils often is processed in order to increase the content of EPA and/or DHA to suitable levels and the removal or reduction of cholesterol have the potential to substantially increase
15 marketability and value. Therefore, the present invention also discloses a health supplement and a pharmaceutical respectively, containing at least a marine oil, such as fish oil, which marine oil is prepared according to the previously mentioned process, in order to decrease the
20 total amount of cholesterol present in the marine oil. It shall be noted that the invented process may also be used for marine oils which has not been processed in order to increase the content of EPA and/or DHA to suitable levels.

25 In another embodiment of the invention the pharmaceutical and/or health supplement is preferably intended for treating cardiovascular diseases (CVD) and inflammatory diseases, but they also have positive effects on other CVD risk factors such as the plasma
30 lipid profile, hypertension and vascular inflammation. In more preferred embodiment of the invention the pharmaceutical and/or health supplement comprises at least one of EPA/DHA triglycerides/ethyl esters and is

intended for a range of potential therapeutic applications including; treatment of hypertriglyceridaemia, secondary prevention of myocardial infarction, prevention of atherosclerosis, treatment of hypertension, mental disorders and/or kidney disease and to improve children's learning ability.

Preferably, the pharmaceutical and/or health supplement prepared according to at least one of the previously mentioned processes is based on fish oil.

Further, the present invention also disclose a marine oil product, prepared according to at least one of the previously mentioned processes. Preferably, the marine oil product is based on fish oil or a fish oil composition.

In another preferred embodiment the stripping process is followed by a trans-esterification process. Preferably, the stripping processing step is followed by the steps of; subjecting the stripped marine oil mixture to at least one trans-esterification reaction with a C_1-C_6 alcohol under substantially anhydrous conditions, and in the presence of a suitable catalyst (a chemical catalyst or an enzyme) to convert the fatty acids present as triglycerides in the marine oil mixture into esters of the corresponding alcohol, and thereafter subjecting the product obtained in the step above to at least one or more distillations, preferably one or more molecular distillations.

After the trans-esterification reaction some glycerides and most of the bound cholesterol will remain unreacted. Both the unreacted glycerides and the bound (esterified) cholesterol will have higher boiling points than the valuable esters of polyunsaturated fatty acids, and will therefore be concentrated in the residue (waste)

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fraction. Thereby a substantial reduction in bound cholesterol can be obtained in the distillate (product) fraction..

By combining the steps of first stripping the cholesterol in free form from the marine oil triglycerides using a volatile working fluid, followed by catalysed esterification of the marine oil with a C₁-C₆ alcohol under substantially anhydrous conditions, and thereafter distillation under conditions suitable to enrich the bound cholesterol in the residium (waste) fraction, a fatty acid ester product with a significant reduction in both free and bound cholesterol can be produced. More preferably, said C₁-C₆ alcohol is ethanol.

In another preferred embodiment of the invention the volatile working fluid comprises at least one of an ester, amides and/or esters composed of longer fatty acids and shorter alcohols or amines, or any combination thereof.

20 BRIEF DESCRIPTION OF THE DRAWINGS

The advantages and details of the present invention will become apparent from the following description when taken in conjugation with the accompanying drawings, in which;

25 FIG 1 is a schematic flow chart of one embodiment illustrating a method for decreasing the amount of cholesterol in a marine oil, by adding a volatile working fluid prior to a molecular distillation.

30 DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

A number of preferred embodiments of the process for decreasing the amount of cholesterol in a mixture com-

prising a volatile working fluid and a marine oil, containing the cholesterol, will be disclosed below.

A first embodiment of a process for decreasing the amount of cholesterol in a marine oil by adding a volatile working fluid prior to a molecular distillation is presented in figure 1. The starting marine oil in the first embodiment of the invention is a fish oil whether freshly refined, reverted or mixtures thereof characterized by an initial or native cholesterol level. The exact amount of cholesterol varies depending upon such factors as fish species, seasonality, geographical catch location and the like.

As used herein the term molecular distillation is a distillation process performed at high vacuum and preferably low temperature (above 120°C). Herein, the condensation and evaporation surfaces are within a short distance from each other, so as to cause the least damage to the oil composition.

The molecular distillation plant (1) illustrated in figure 1, comprises a mixer (2), a pre-heater (3), a degasser (4), a distillation unit (5) and a vacuum pump (6). In accordance with this embodiment, a volatile working fluid comprising an ethyl ester fraction (6% relative to the oil) is added to a fish oil mixture and blended in a mixer (2). The fish oil mixture is then optionally passed through a means for controlling the oil feed rate, such as an ordinary throttling valve. The fish oil mixture is then preheated with a heating means (3) such as a plate heat exchanger to provide a preheated fish oil mixture. The mixture is then passed through a degassing step (4) and admitted into the short path evaporator (5), a tube (7) including the condensation (8) and evaporation (9) surface. The fish oil mixture to be concentrated is

picked up as it enters the tube (7a) by rotating blades (not shown). The blades extend nearly to the bottom of the tube and mounted so that there is a clearance of about 1,3 mm between their tips and the inner surface of the tube. In addition, the blades are driven by an external motor. The fish oil mixture is thrown against the tube wall and is immediately spread into a thin film and is forced quickly down (A) the evaporation surface. The film flows down by gravity, and as it falls the light and heavy fractions are separated because of differences in boiling point.

Heated walls and high vacuum strips off the volatile working fluid together with the cholesterol, i.e. the more volatile components (distillate) is derived to the closely positioned internal condenser (8), the less volatile components (residue) continues down the cylinder. The resulting fraction, the stripped fish oil mixture containing at least the fatty acids EPA and DHA is separated and exit through an individual discharge outlet (10).

In a second embodiment a falling film evaporator is used. In falling film evaporators liquid and vapours flow downwards in parallel flow. The liquid to be concentrated, herein the fish oil mixture, is preheated to boiling temperature. The oil mixture enters the heating tubes via a distribution device in the head of the evaporator, flows downward at boiling temperature, and is partially evaporated. This gravity-induced downward movement is increasingly augmented by the co-current vapour flow. Falling film evaporators can be operated with low temperature differences between the heating media and the boiling liquid, and they also have short product contact times, typically just a few seconds per pass.

In a third embodiment of the invention the process is carried out by a short path distillation, which includes the use of a short path evaporator that integrates the features and advantages of thin film or wiped film evaporators but adds internal condensing for applications. Short path evaporators are widely used in fine and specialty chemicals for thermal separation of intermediates, concentration of high value products, and molecular distillation under fine vacuum conditions. Their key features make them uniquely suitable for gentle evaporation and concentration of heat sensitive products at low pressures and temperatures.

In a fourth embodiment of the invention the stripping process is followed by the steps of subjecting the stripped marine oil mixture to at least one transesterification reaction with a C₁-C₆ alcohol under substantially anhydrous conditions and thereafter subjecting the product obtained in the step above to at least one or more distillations, preferably one or more molecular distillations. The key step in all transesterification reactions is the reaction between an ester mixture, composed of fatty acids bound to an alcohol A, and an alcohol B where the reaction products are an ester mixture, composed of the same fatty acids bound to alcohol B, and alcohol A as shown in this general formula:



The reaction is preferably catalysed and the reaction is an equilibrium and the yield of the expected fatty acid ester is to a large extent controlled by the concentration of the alcohols. Herein, for instance the

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stripping process is followed by a catalysed trans-esterification of marine oil triglycerides. The separation of the ethyl ester fraction from the fraction containing the unreacted glycerides and bound cholesterol is suitably carried out by at least one of a molecular distillation technique, whereby the less volatile residual mixture can be readily removed from the relatively volatile ethyl esters.

It should be understood that many modifications of the above embodiments of the invention are possible within the scope of the invention such as the latter is defined in the appended claims. It will be apparent for one skilled in the art that various changes and modifications, i.e. other combinations of temperatures, pressures, and flow rates during the stripping process can be made therein without departing from the spirit and scope thereof.

EXAMPLES

The invention will now be illustrated by means of the following non-limiting example. This example is set forth merely for illustrative purposes and many other variations of the process may be used. The example below summarize some results from different purification of fish oils by molecular distillation.

EXAMPLE 1

A stripping process for decreasing the amount of cholesterol present in a fish oil mixture in free form with respect to without using a volatile working fluid

This example shows an industrial scale process for decreasing the amount of cholesterol in a refined fish

oil mixture in free form, with and without adding a volatile working fluid to the fish oil mixture, and subjecting the mixture to a molecular distillation process.

Herein, an Anchovy oil from Peru, with a fatty acid composition of 18% EPA and 12% DHA was used. The oil contains about 9 mg cholesterol/g fish oil, of which 6 mg/g was constituted by cholesterol present in free form and about 3 mg/g in bound form. In tests 1 and 3 a volatile working fluid constituted by a fatty acid ethyl ester mixture, 6% ethyl ester relative to the fish oil, i.e. the ratio of (volatile working fluid):(fish oil) about 6:100, was added to the fish oil mixture before subjecting the mixture to a molecular distillation process. All tests below were carried out at mixture flow rates of 900 or 400 kg/h in a molecular distillation unit with an evaporation surface of 11m². Test 1 and 2 were carried out at a temperature of 210°C and at a mixture flow rate of 900 kg/h. Test 3 and 4 were carried out at a lower temperature, 205°C, and at a lower flow rate, 400 kg/h.

The amount of cholesterol present in the fish oil mixture in free form was analysed by a method based on standard high performance liquid chromatographic analyses.

Test	Temp. (°C)	Flow rate (kg/h)	% added ethyl ester	Free cholesterol (mg/g)
1	210	900	6	1,4
2	210	900	0	2,4
3	205	400	6	0,2
4	205	400	0	0,4

Table 1: Amounts of cholesterol present in a fish oil in free form after molecular distillation

The results in the table above illustrates that it is possible to decrease (to separate) an amount of free cholesterol in a marine oil more effective by adding a volatile working fluid to a marine oil composition and thereafter subjecting the fish oil composition to a stripping processing step according to the invention. It is important to note that the effect by adding a volatile working fluid to a marine oil composition, before subjecting at least one stripping processing step, is better, and more interesting, when the stripping process is carried out at higher mixture flow rates, preferably flow rate in the interval of 80-150 kg/h·m². Under these conditions, the use of a volatile working fluid opens up for a much better utilization of the capacity of the process equipment and a more rapid stripping process.

Another advantage by using a volatile working fluid according to the invention is that the stripping effect is satisfactory at low temperatures [temperatures in the interval of 120 - 220°C] for marine oils. Namely, for marine oils, such as fish oils, and other temperature accommodating oils (oils comprising long chains of polyunsaturated fatty acids) it is important to keep the temperature load during the processes as low as possible. But, this is less important for other oils not mentioned above.

Further, the effect of adding a volatile working fluid, compared with no adding of the same, is less noticeable in the case when the stripping process is carried out at low mixture flow rates [i.e. flow rates < 30 kg/h·m²]. But on the other hand, it is not known commercially interesting to carry out a stripping process using low feed rates and relatively high temperatures because the stripping process will take too long to finish. Addi-

tionally, today it is a problem for the marine oil industry to find effective and rapid techniques that are able to decrease the amount of cholesterol in marine oils at higher flow rates.

5 The tests above also show that the amount of free cholesterol is reduced from about 6 mg/g to about 1,4 mg/g by adding a volatile working fluid to a fish oil mixture prior to a molecular distillation process, which process being carried out at a temperature of 210°C and
10 at a mixture flow rate of 900 kg/h pr. Here, the amount of cholesterol in free form is decreased with about 75-80%.

 When the stripping process is carried out at 900 kg/h the amount of free cholesterol is reduced further
15 compared to the stripping process where no ethyl ester (working fluid) has been added, at the same flow rate. Note that the content of bound cholesterol is less affected by the stripping process according to the invention. Additionally, the use of very high temperatures,
20 i.e. temperatures above 270°C, isn't of interest. Such temperatures will cause damage to the oil. Too high temperatures also can be harmful for the production equipment.

 Further, the amount (%) of addition of ethyl ester
25 is also of importance. Addition of at least 4% ethyl ester or an ethyl ester fraction has also generated good results. Preferably, the ratio of (volatile working fluid):(marine oil) is about 1:100 to 15:100 and more preferably, the ratio of (volatile working fluid):(marine
30 oil) is about 3:100 to 8:100.

 While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent for one skilled in the art that various

change and modifications can be made therein without departing from the spirit and scope thereof.

DEFINITIONS

5 As used herein the term marine oil also includes marine fat and a fermented or refined product containing at least n-3 polyunsaturated fatty acids, predominately EPA and DHA from a raw marine oil. Further, the marine oil is preferably oil from at least one of fish, shellfish
10 (crustaceans) and sea mammals, or any combination thereof. Non limiting examples of fish oils are Menhaden oil, Cod Liver oil, Herring oil, Capelin oil, Sardine oil, Anchovy oil and Salmon oil. The fish oils mentioned above may be recovered from fish organs, e.g. cod liver
15 oil, as well as from the meat of the fish, from the whole fish or from fish waste. Additionally, the term "oil and fat" means fatty acids in at least one of the triglyceride and phospholipid forms. Generally, if the start material in the stripping process is a marine oil,
20 the oil may be any of raw or partially treated oil from fish or other marine sources and which contains fatty acids, including polyunsaturated fatty acids, in the form of triglycerides. Typically, each triglyceride molecule in such a marine oil will contain, more or less randomly,
25 different fatty acid ester moieties, be the saturated, monounsaturated or polyunsaturated, or long chain or medium chain. Further, examples of vegetable oils or fats are corn oil, palm oil, rapeseed oil, soybean oil, sunflower oil and olive oil. Additionally, the marine fat or
30 oil may be pre-processed in one or several steps before constituting the start material in the stripping process as described above.

As used herein the term edible means edible for humans and/or animals. Additionally, as used herein the term "for use in cosmetics" means an oil or a fat that can be used in products that contributes to enhance humans appearance and/or health, e.g. cosmetic and/or beauty care products. Further, a fat or an oil, being edible or for use in cosmetics, according to the invention can also be a blend of e.g. microbial oils, fish oils, vegetable oils, or any combination thereof.

As used herein the term microbial oils also includes "single cell oils" and blends, or mixtures, containing unmodified microbial oils. Microbial oils and single cell oils are those oils naturally produced by microorganisms during their lifespan.

As used herein the term working fluid is interpreted to include a solvent, a solvent mixture, a composition and a fraction, e.g. a fraction from a distillation process, that has a suitable volatility, comprising at least one of esters composed of C10-C22 fatty acids and C1-C4 alcohols, amides composed of C10-C22 fatty acids and C1-C4 amines, C10-C22 free fatty acids, mineral oil, hydrocarbons and bio-diesel.

As used herein the term essentially equally or less volatile is interpreted to include that the volatile working fluids having a suitable volatility in relation to the volatility of the cholesterol present in the marine oil in free form that is to be stripped off from the marine mixture. Further, commonly this is the case when the volatility of the working fluid is the same or lower than the volatility of the cholesterol present in free form. However, the term essentially equally or less volatile is also intended to include the case when the vola-

tile working fluid is somewhat more volatile than the cholesterol in free form.

Further, as used herein the term stripping is interpreted to include a general method for removing, separating, forcing or flashing off gaseous compounds from a liquid stream. In addition, the term "stripping processing step" preferable herein is related to a method/process for decreasing the amount of cholesterol in a marine oil or fat by one or more distilling or distillation processes, e.g. short path distillations, thin-film distillations (thin-film stripping or thin-film (steam) stripping), falling-film distillations and molecular distillations, and evaporation processes.

As used herein the term "together with", means that the volatile working fluid will be stripped off together with, combined with, or adhering the cholesterol, namely that the cholesterol will accompany the working fluid.

As used herein the term health supplement is interpreted to include food and food supplement to animals and/or humans, fortification of food, dietary supplement, functional (and medical) food and nutrient supplement.

As used herein the term "treating" means both treatment having a curing or alleviating purpose and treatment having a preventive purpose. The treatment can be made either acutely or chronically. In addition, as used herein the term pharmaceutical means pharmaceutical preparations and compositions, functional food (foodstuff having an increased value) and medical food. A pharmaceutical preparation according to the present invention may also comprise other substances such as an inert vehicle or a pharmaceutically acceptable adjuvance, carriers, preservatives etc., which all are well-known to those skilled in the art.

As used herein the term "oils with a low quality" preferably means that the oil contains high amounts of free fatty acids, that makes them less useful for nutritional purposes and that traditional alkaline refining in such oils is complicated and costly. Additionally, as used herein, the term mineral oil is interpreted to include mineral oil products such as e.g. fractions from distillation processes and white spirit. As used herein hydrocarbons is interpreted to include organic compounds, that are relatively large molecules composed mainly of carbon and hydrogen. They can also include nuclei of nitrogen, phosphorus, sulphur, and chlorine, among others.

Further, the method according to the invention is also applicable to a variety of sterols including cholesterol. Most of these sterols can, when present on free form, be separated from a marine oil by the described technique as long as the volatile working fluid is essentially equally or less volatile than the sterol in free form that is to be separated from the marine oil mixture.